



# **Functional studies of the BiP chaperone protein and its role in protein translocation.**

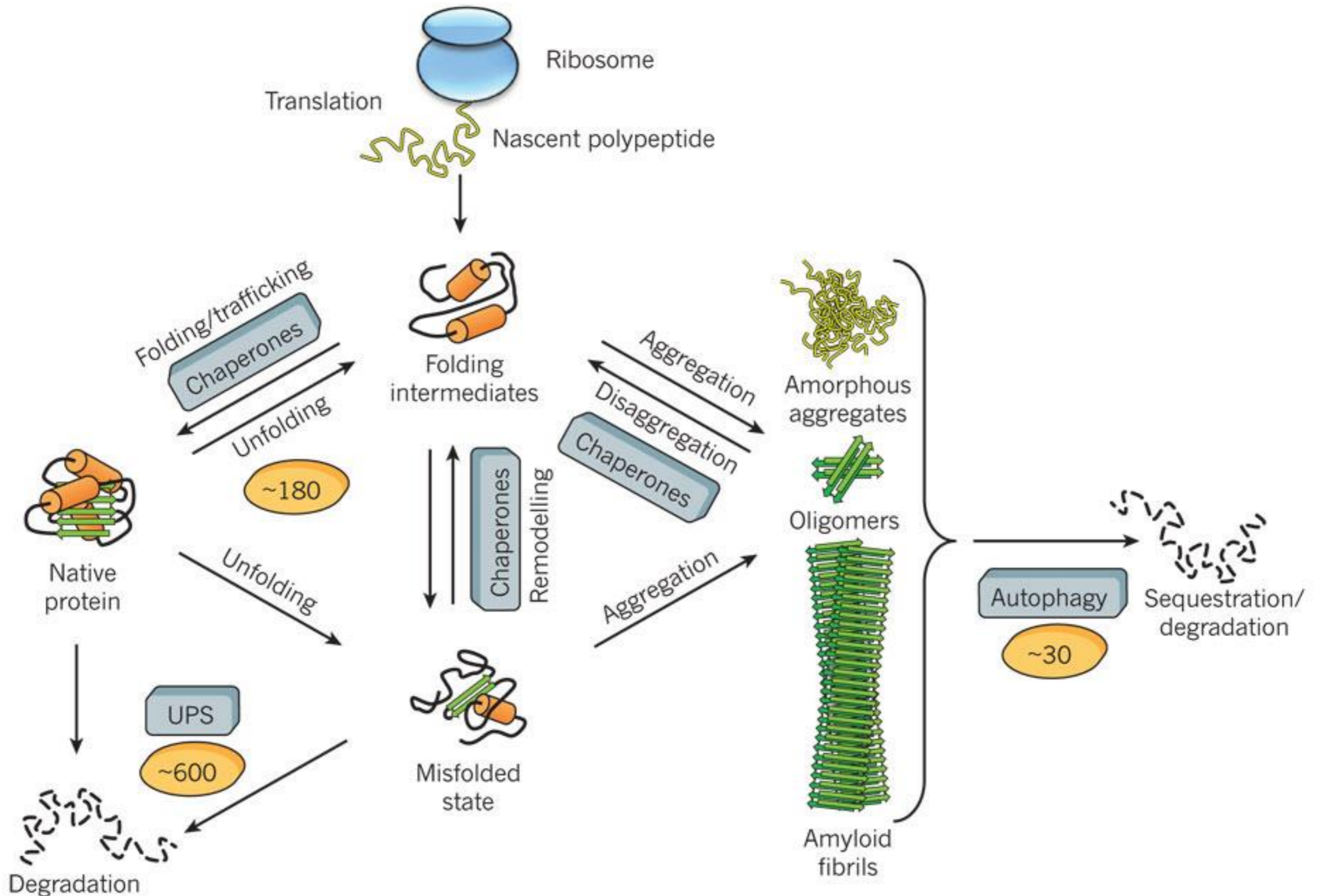
Christian A.M. Wilson

Assistant professor

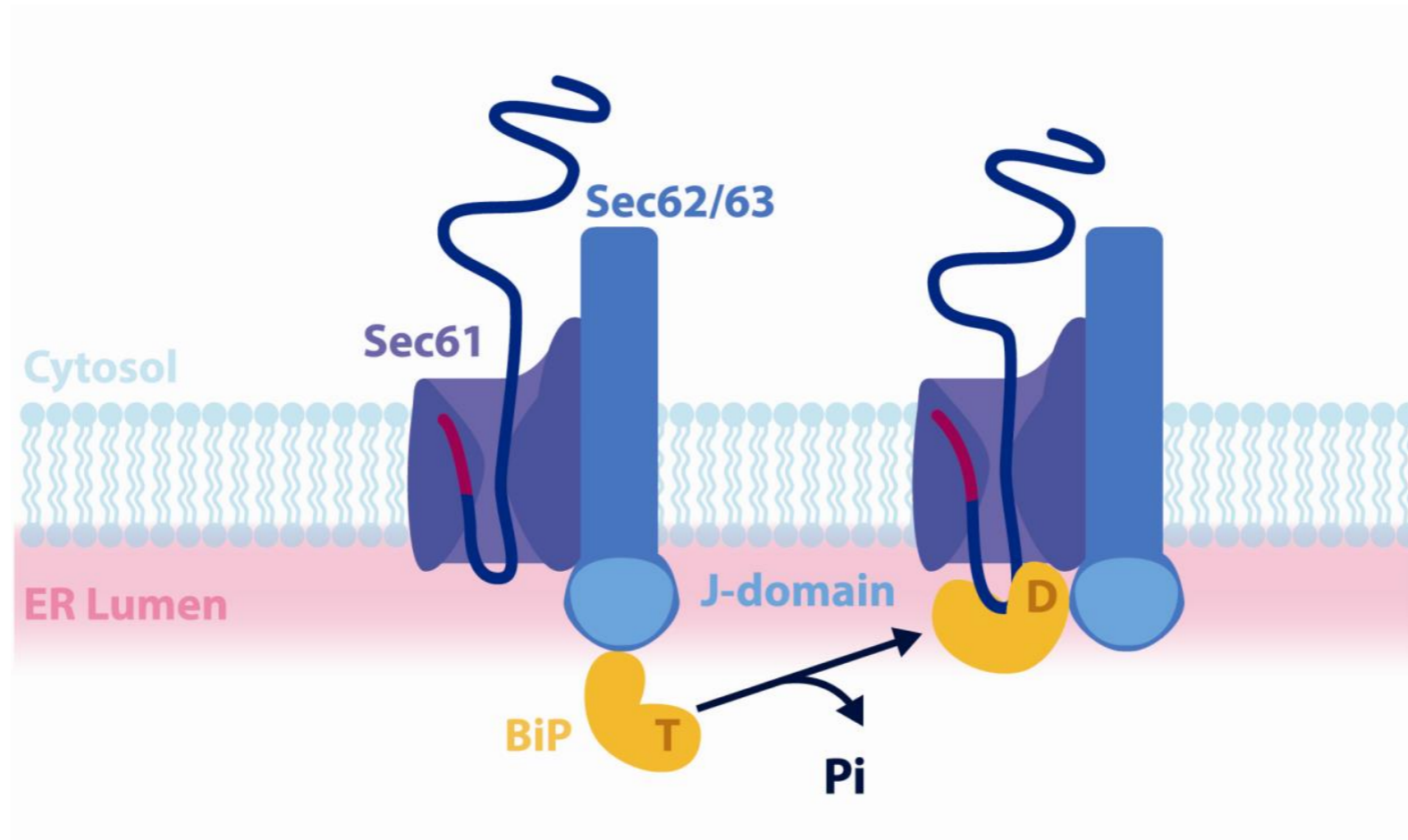
Biochemistry laboratory, Biochemistry and Molecular Biology department, Faculty of Chemical Sciences and Pharmacy, Universidad De Chile

TYAN, Brazil, August 2017

# Proteostasis: Life cycle of a protein



# Post-translation translocation

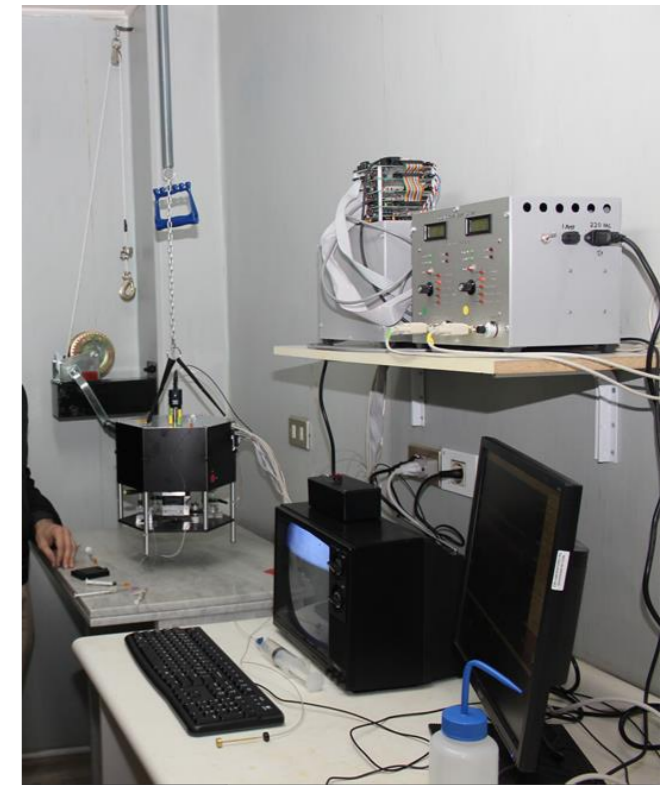
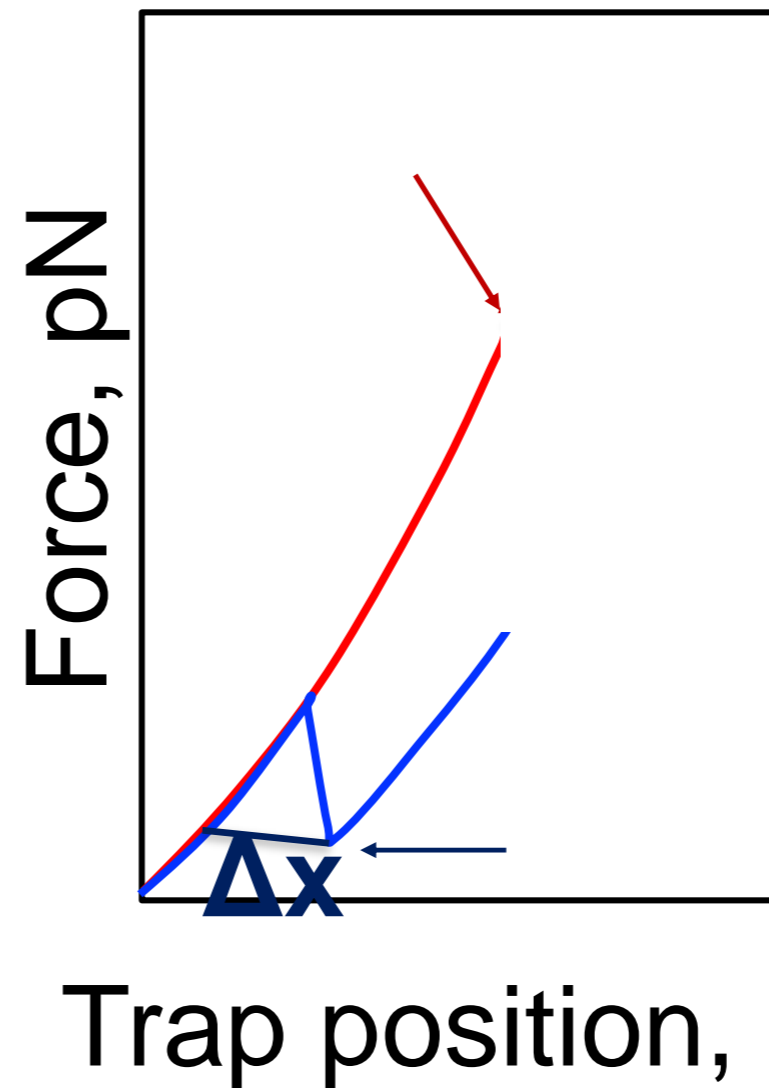
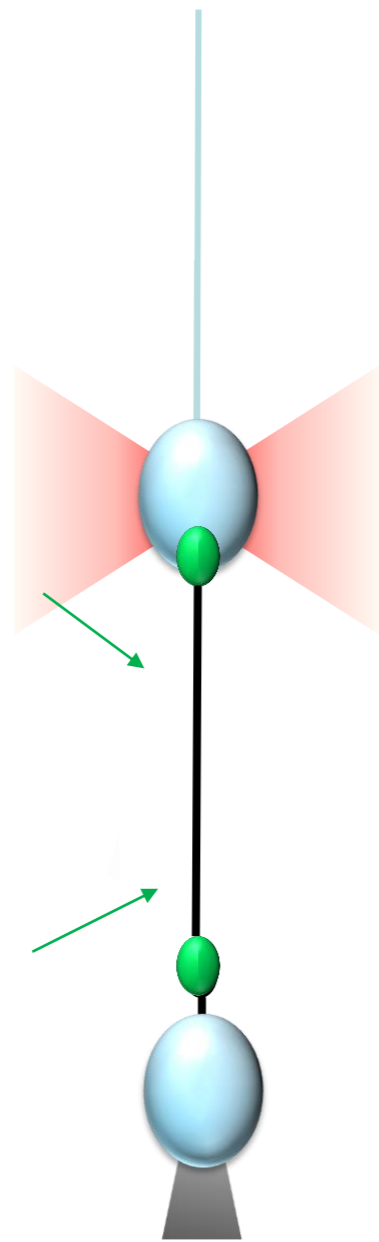


How does BiP (Hsp70) chaperone work?

Modified from Park and Rapoport (2012).

# Optical tweezers experiments

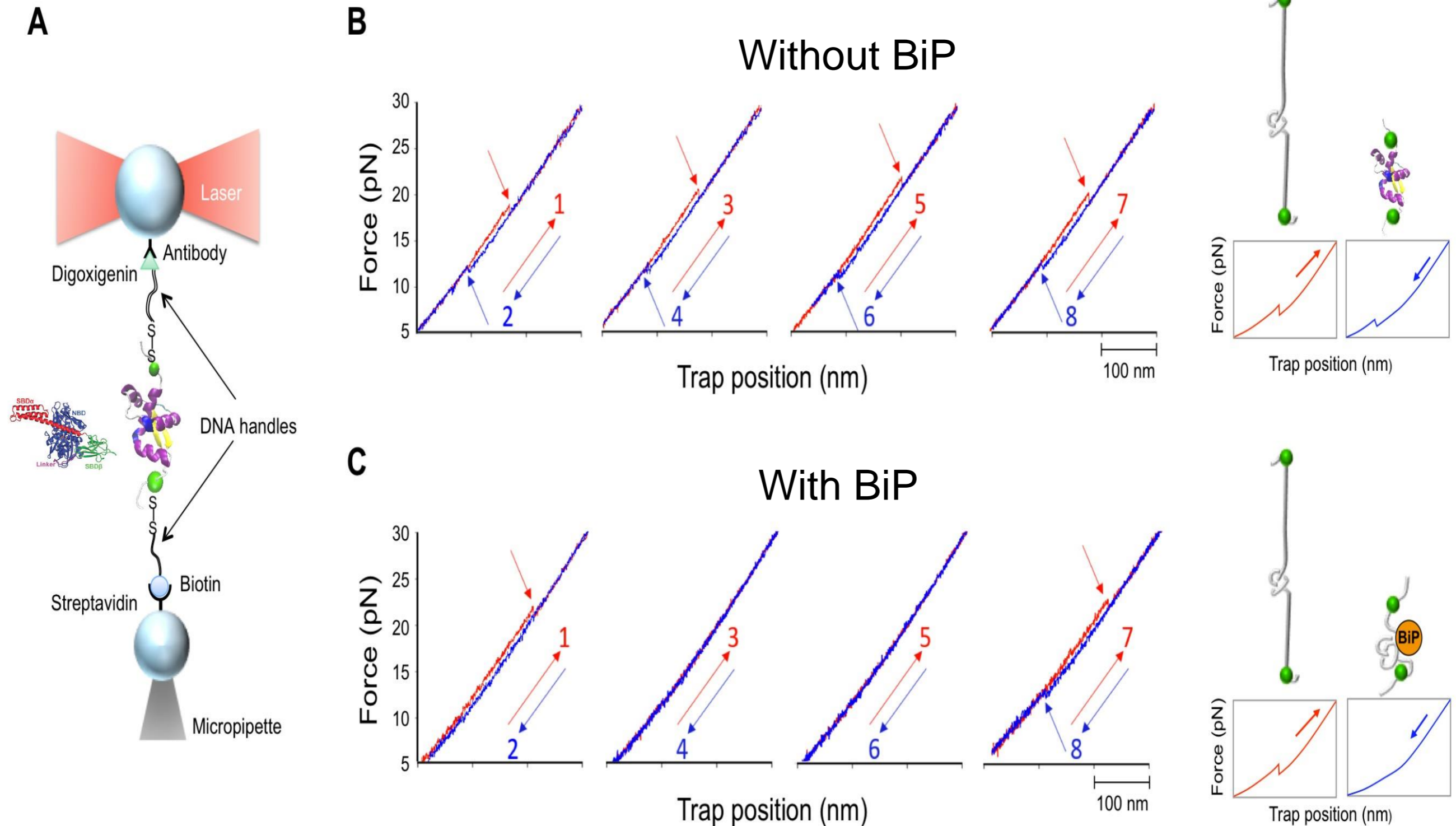
## Force ramp



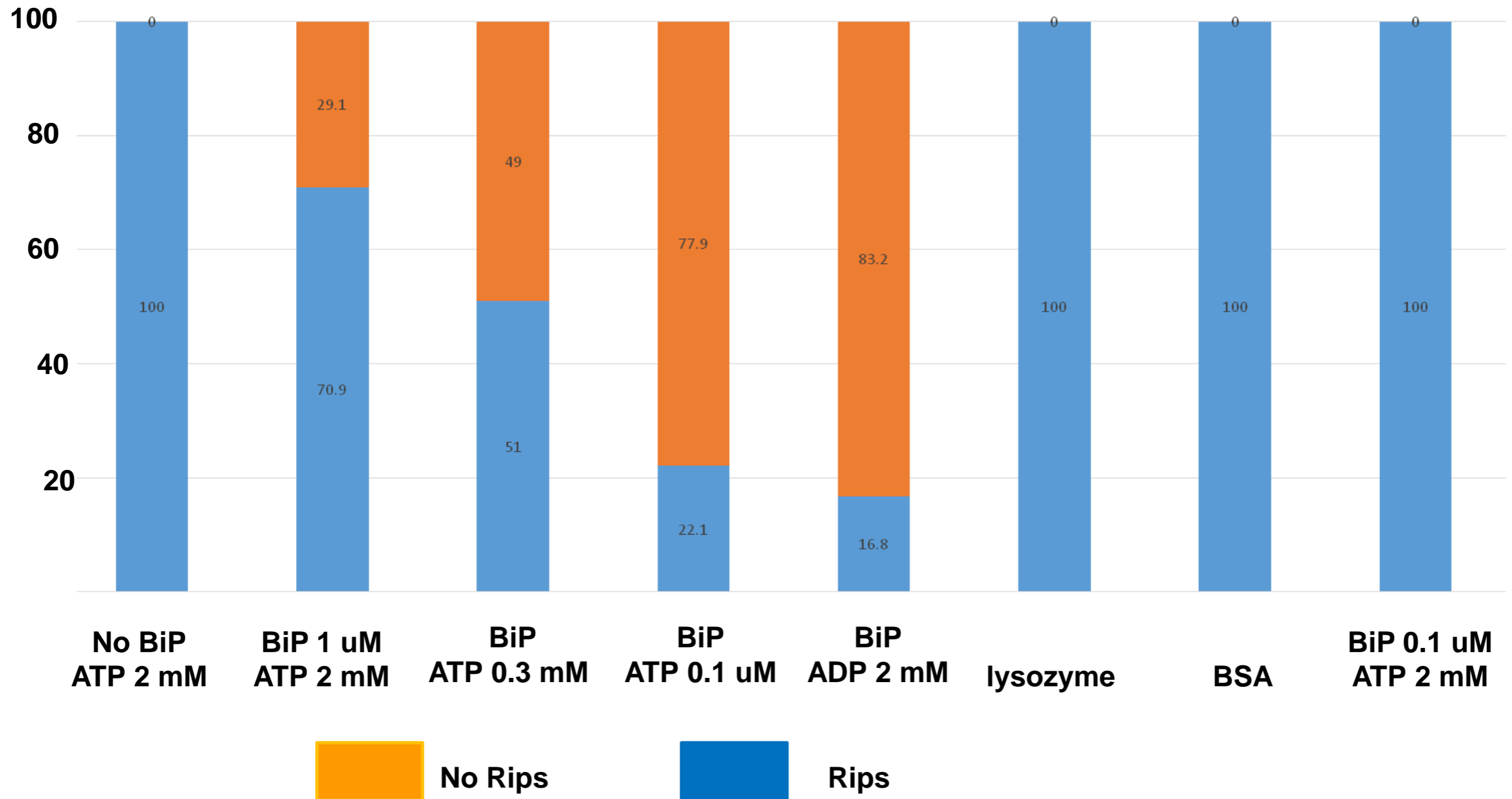
Chilean optical tweezers

Animation provided by Maira Rivera

# Effect of BiP chaperone in protein folding at single molecule level



# Refolding rip percentage as function of nucleotide type and concentration



# Binding parameters fro BiP with differents nucleotides

**Table I.** *Kinetic Parameters for BiP in Different Conditions*

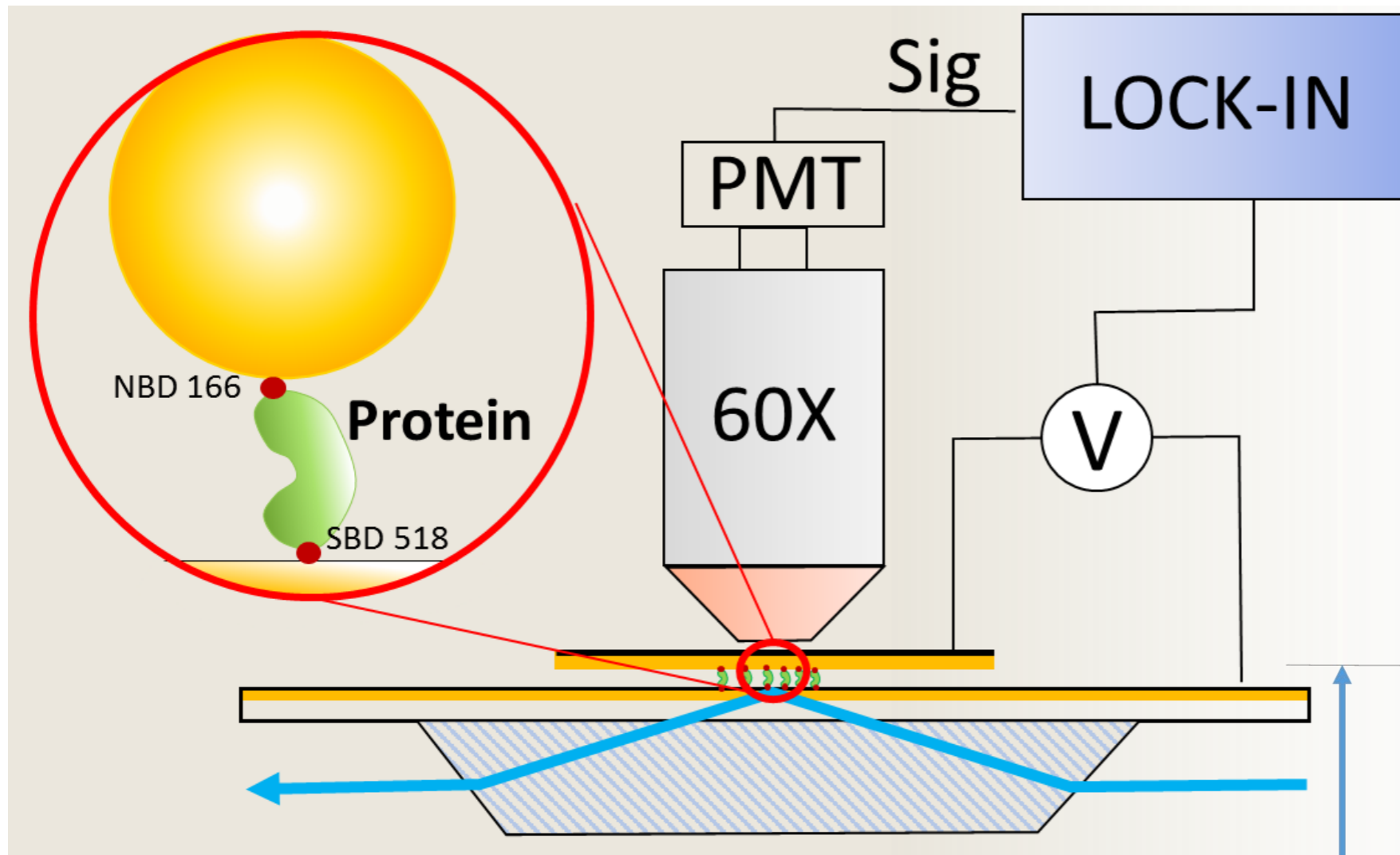
Condition	app $K_D$ ( $\mu M$ ) <sup>a</sup>	app $K_D$ ( $\mu M$ ) <sup>b</sup>	$k_{on}$ ( $\mu M^{-1} \text{ sec}^{-1}$ )	$k_{off}$ ( $\text{sec}^{-1}$ )
1 $\mu M$ BiP 2 mM ATP	$3.37 \pm 0.70$	$1.75 \pm 0.43$	$0.012 \pm 0.0022$	$0.021 \pm 0.0034$
1 $\mu M$ BiP 0.33 mM ATP	$1.43 \pm 0.25$	$1.31 \pm 0.50$	$0.013 \pm 0.0035$	$0.017 \pm 0.0046$
1 $\mu M$ BiP 0.1 $\mu M$ ATP	$0.35 \pm 0.06$	$0.33 \pm 0.15$	$0.030 \pm 0.0098$	$0.0099 \pm 0.0032$
1 $\mu M$ BiP 2 mM ADP 0.33 mM ATP	$0.24 \pm 0.05$	$0.28 \pm 0.12$	$0.029 \pm 0.0082$	$0.0080 \pm 0.0025$

<sup>a</sup> app  $K_D$  calculated with Eq. (2).

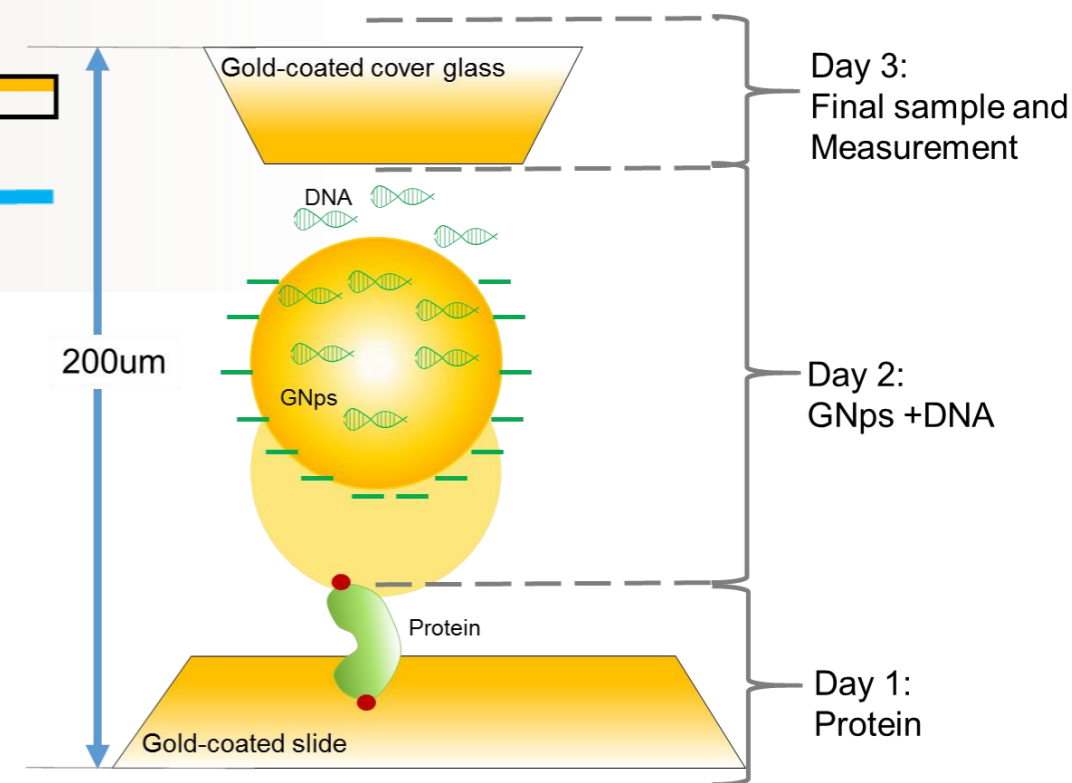
<sup>b</sup> app  $K_D = k_{off}/k_{on}$ .

Ramírez et al. Protein Sci., 2017

# Viscoelasticity measurement of BiP by nanorheology

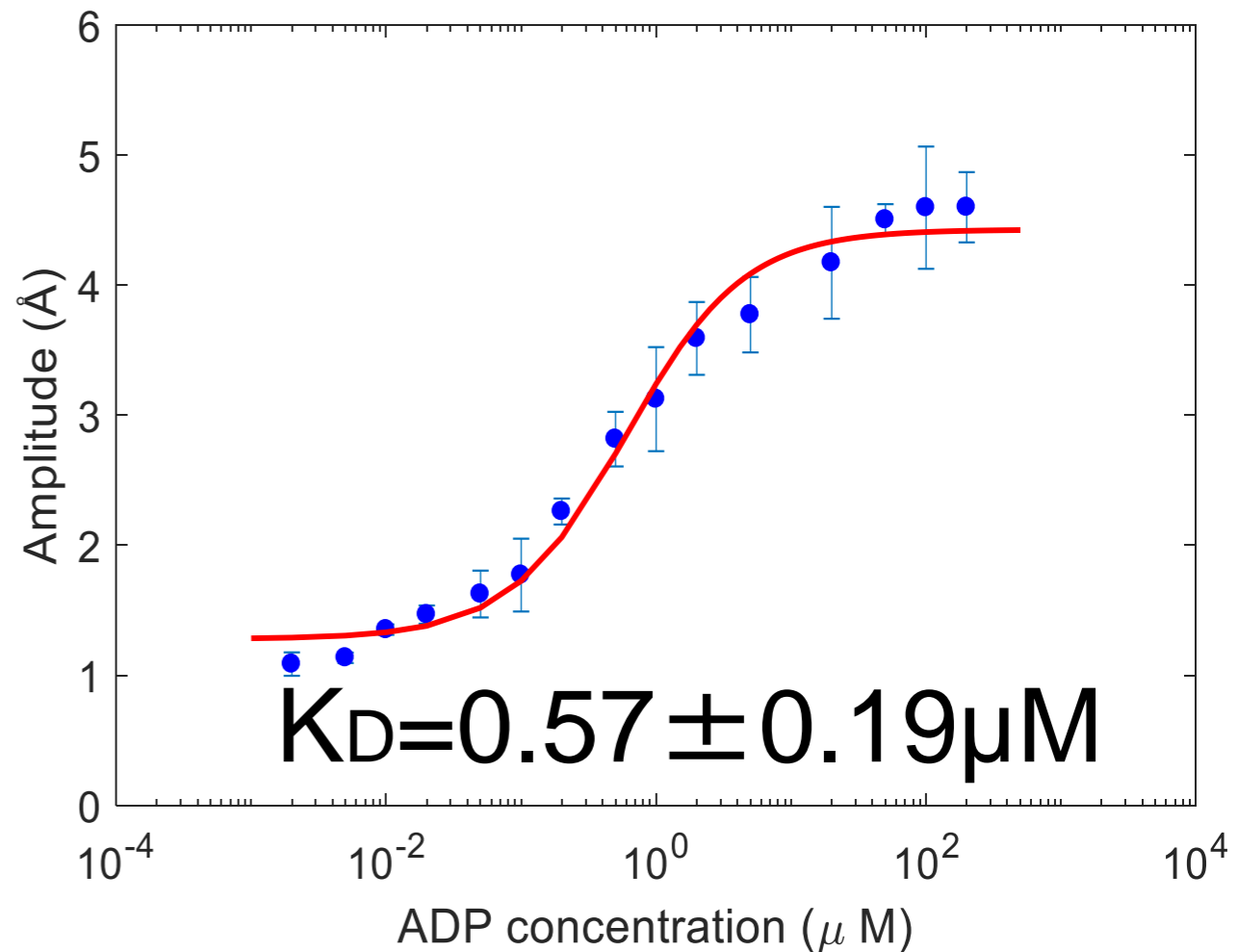
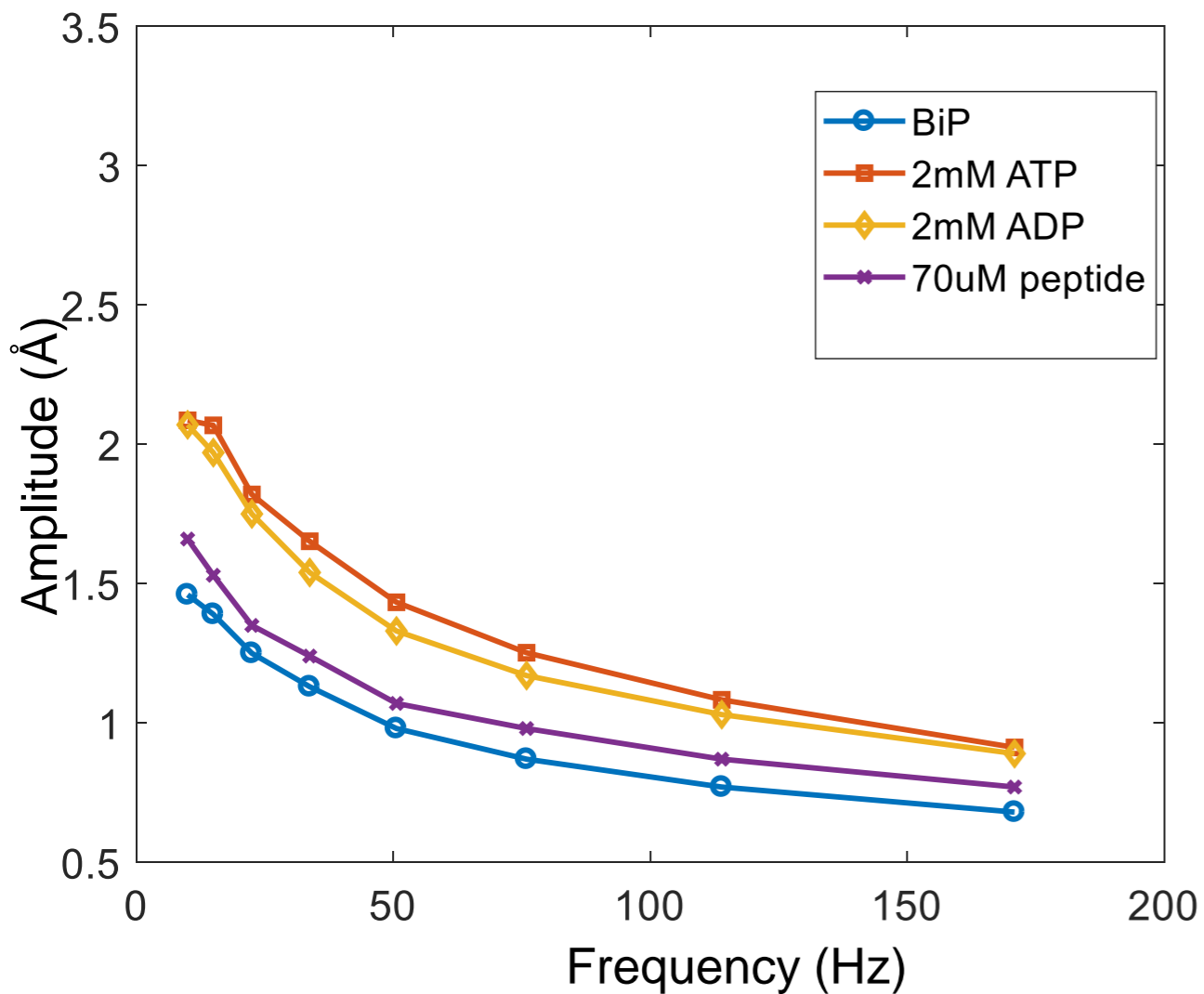


Mechanical response, parameters of dissipation and elasticity of the **BiP** protein.





# Viscoelasticity measurement of BiP by nanorheology

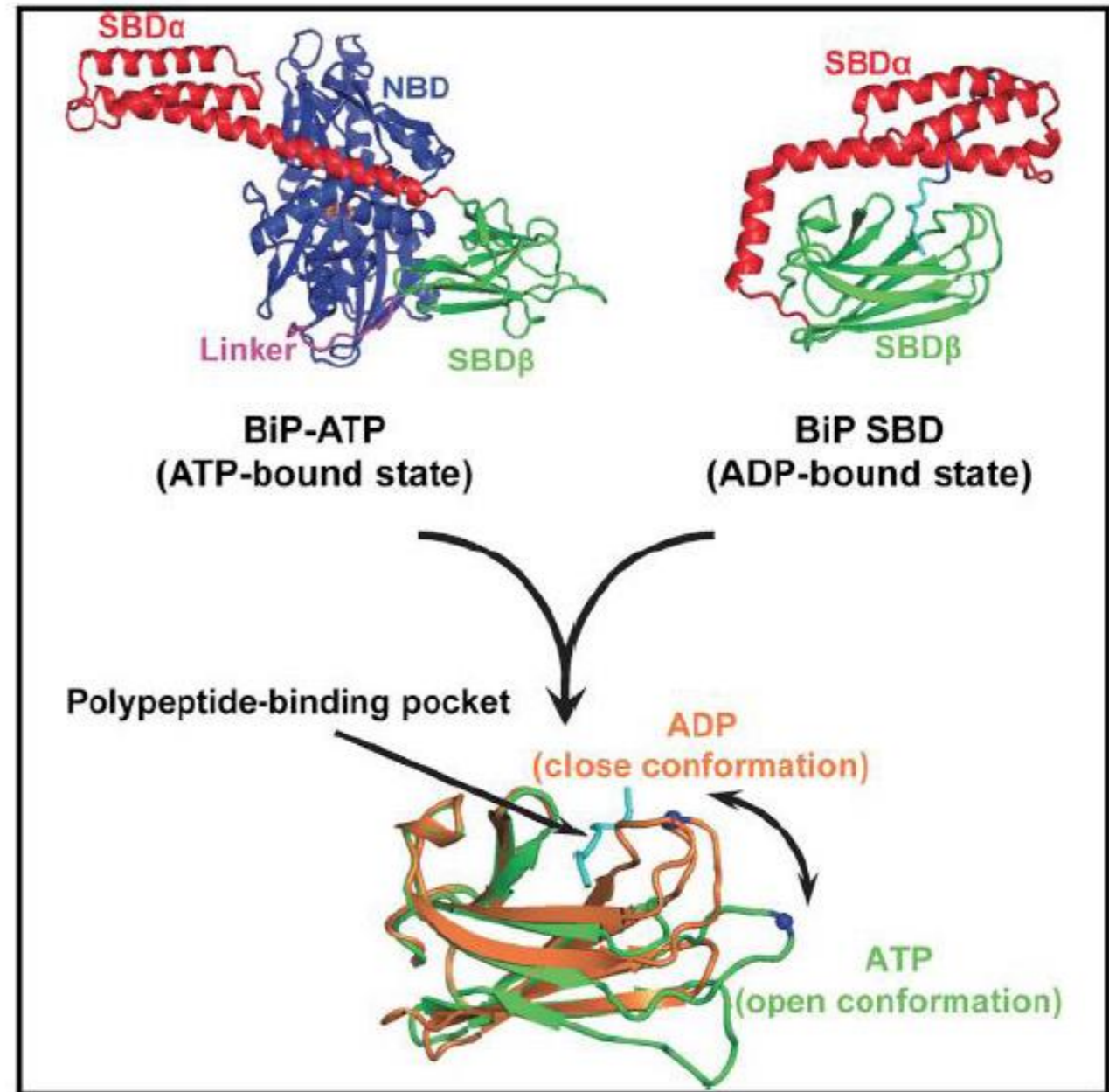
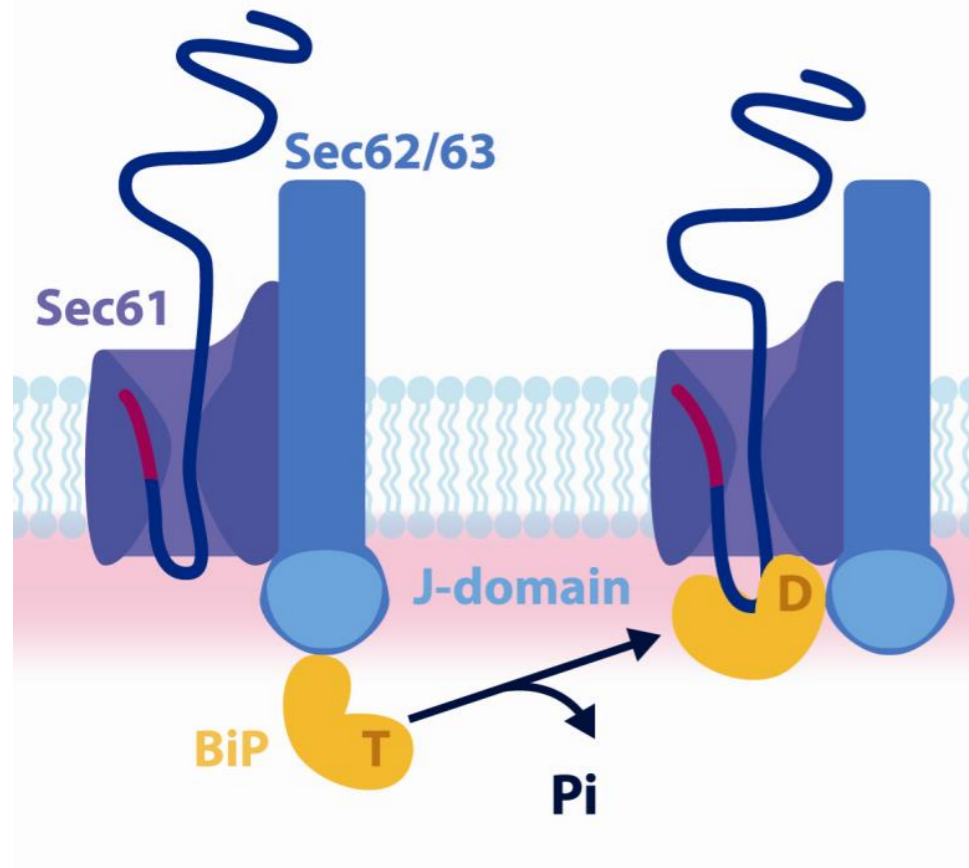


# Viscoelasticity measurement of BiP by nanorheology

substrate	K <sub>D</sub> (μM)	
	experiment	literature
ADP	0.57 ± 0.19	0.29 <sup>1</sup>
ADP+1.8μM peptide	0.10 ± 0.07	0.24 <sup>2</sup>
ATP	0.97 ± 0.20	0.20 <sup>1</sup>
AMP PNP	0.48 ± 0.10	
AMP PNP +1.8μM peptide	0.003 ± 0.002	

1. Marcinowski et al. Nat. Struct. Mol. Biol., 2011
2. Ramírez et al. Protein Sci., 2017

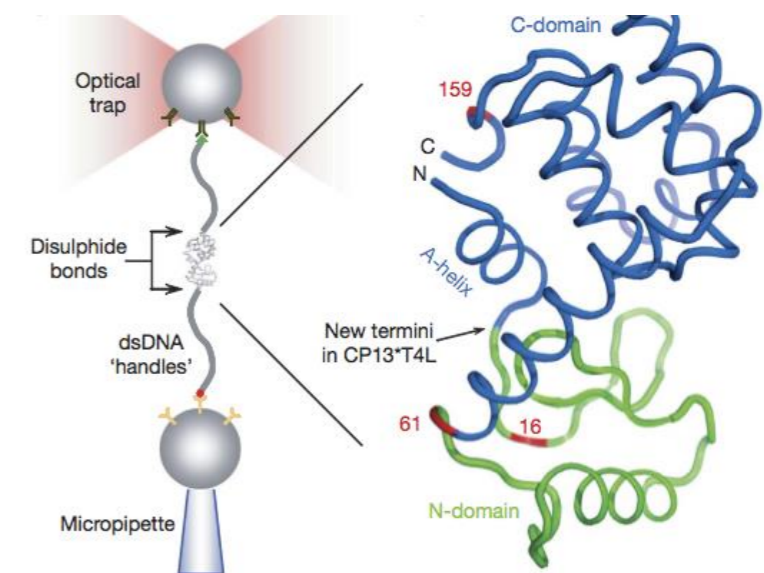
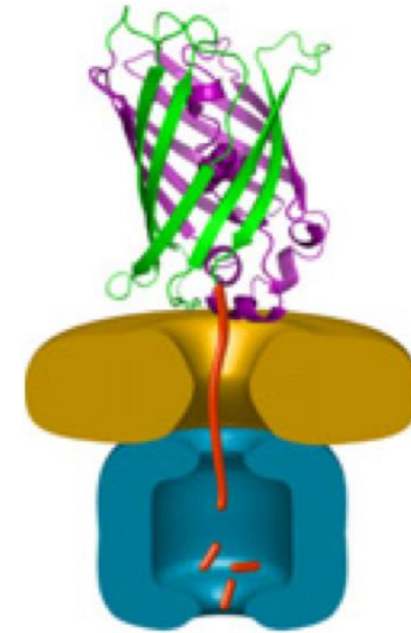
# Mechanism of BiP action



3D structure of BiP

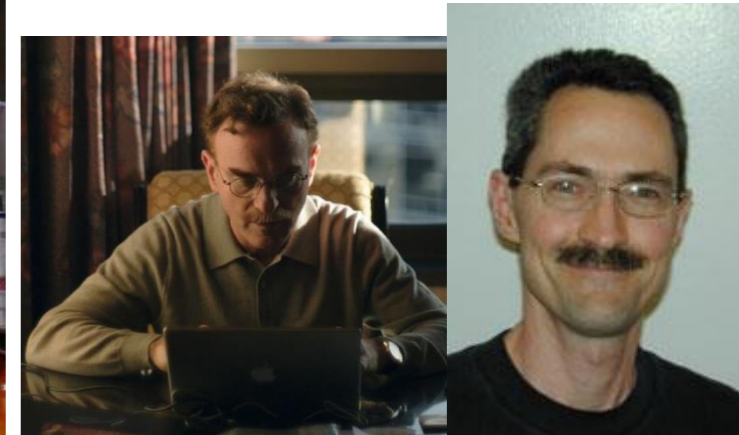
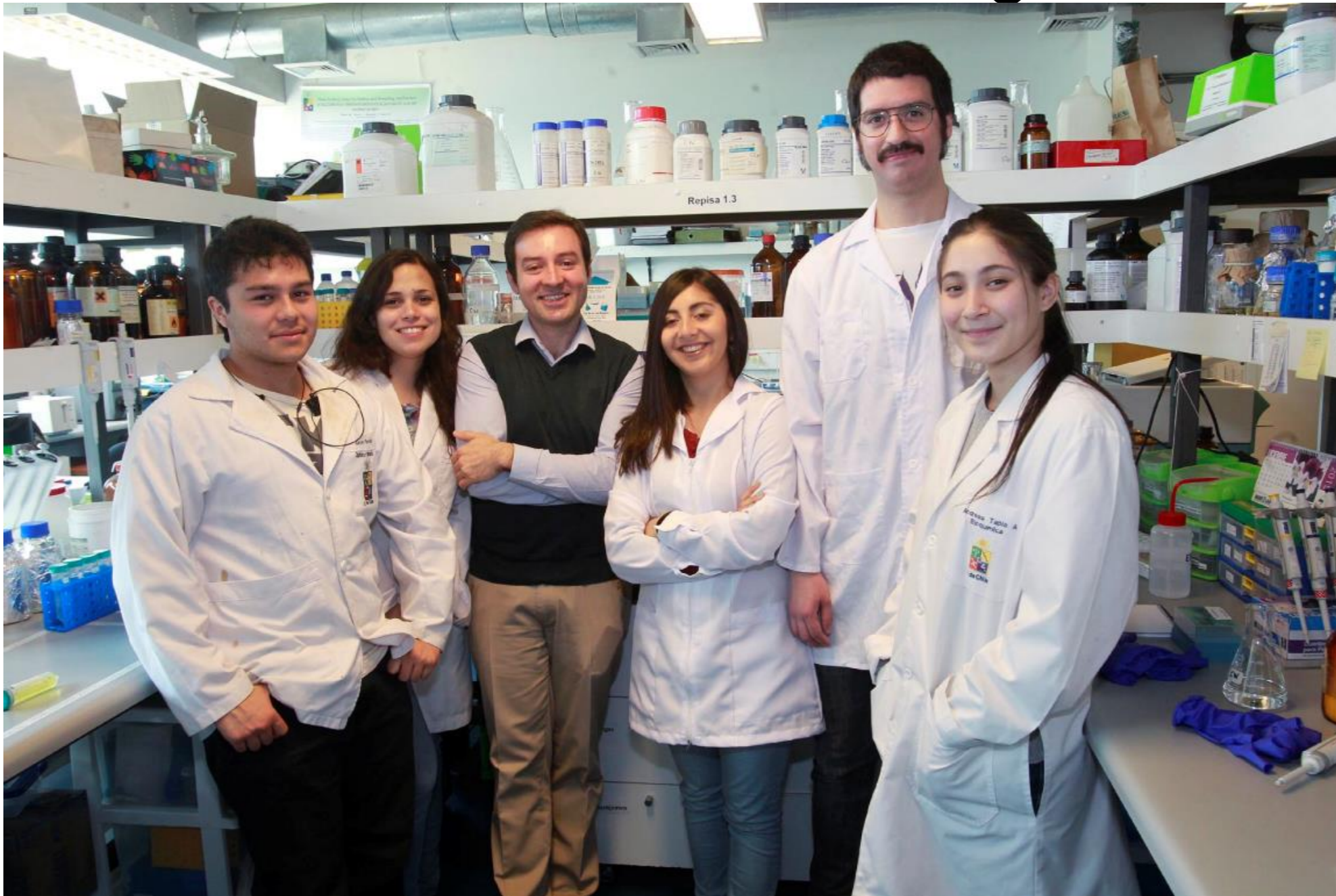
# Proteins exert and respond to force

- Proteins participate in many force-driven cellular processes
  - Movement
  - Transmembrane transport
  - Enzyme catalysis
  - Forced denaturation
- Force is becoming a useful tool for studying protein folding



Maillard et al. Cell, 2011  
Shank et al. Nature, 2010

# Acknowledgments



Former members

- Marcela Vega
- Mauricio Valdivia
- Andreas Tapia
- Carolina Ramírez
- Mauricio Morales

Fondecyt  
U-inicia  
U-redes PCI